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(54) Title: HUMAN CHITINASE IMMUNOGLOBULIN FUSION PROTEINS

(57) Abstract: Chitinase immunoglobulin fusion products with unexpectedly improved serum half-life and improved formulation properties are provided.

CHITINASE IMMUNOGLOBULIN FUSION PRODUCTS

FIELD OF THE INVENTION

The present invention relates generally to chitinase immunoglobulin fusion products comprising chitinase or fragments or variants thereof, and uses of such products.

BACKGROUND

Chitin is a linear homopolymer of β-(1,4)-linked N-acetylglucosamine residues. This polysaccharide is second only to cellulose as the most abundant organic substance. The exoskeleton of arthropods is composed of chitin. In addition, fungi and other parasites contain chitin in their outer cell wall, where it serves important structural and protective roles. Disruption of the fungal cell wall and membrane has been a useful therapeutic strategy against fungi and parasites. For example, Amphotericin B and fluconazole exert their antifungal activity by affecting membrane steroids. Despite the existence of antifungal therapeutics, fungal infections of humans have increasingly become responsible for life-threatening disorders. See, Georgopapadakou *et al.*, *Trends Microbiol.*, *3:* 98-104 (1995). The fungal species and parasites responsible for these diseases include *Candida, Aspergillus, Cryptococcus, Histoplasma, Blastomyces, Coccidioides, Paracoccidioides, Fusarium* and *Pneumocystis*. These pathogens are particularly dangerous in immunocompromised individuals, such as patients with AIDS, patients undergoing chemotherapy, and immunosuppressed organ transplant patients.

Chitin can be degraded by the enzyme chitinase. Chitinase enzymes are found in plants, microorganisms, and animals. Bacterial chitinase helps to provide a carbon source for bacterial growth. Insects produce chitinase to digest their cuticle at each molt. In plants, chitinase is thought to provide a protective role against parasitic fungi. Chitinases have been cloned from numerous bacterial [*e.g.*, *Serratia marcescens*, Jones et al., *EMBO J.*, *5*:467-473 (1986)], plant [*e.g.*, tobacco, Heitz et al., *Mol. Gen. Genet.*, *245*:246-254 (1994)], and insect [*e.g.*, wasp, Krishnan et al., *J. Biol. Chem.*, *269*:20971-20976 (1994)] species and have been categorized into two

distinct families, designated family 18 and family 19, based on sequence similarities [Henrissat and Bairoch, *Biochem, J. 293*:781-788 (1993)]. Although the catalytic region of the enzymes in family 18 is largely conserved across numerous species, there is very limited sequence similarity across species for the chitin-binding domain. The only feature common to several family 18 chitin-binding domains is the presence of multiple cysteine residues.

Escott et al., *Infect. Immun., 63*:4770-4773 (1995) demonstrated chitinase enzymatic activity in human leukocytes and in human serum. Overdijk et al., *Glycobiology, 4*:797-803 (1994) described isolation of a chitinase (4-methylumbelliferyl-tetra-N-acetylchitotetraoside hydrolase) from human serum and rat liver. Renkema *et al., J. Biol. Chem., 270*:2198-2202 (February 1995) prepared a human chitotriosidase from the spleen of a Gaucher disease patient. Their preparation exhibited chitinase activity and the article reports a small amount of amino acid sequence of the protein component of the preparation (22 amino terminal residues and 21 residues of a tryptic fragment). The function of human chitinase is also unknown, but a relationship with the pathophysiology of Gaucher disease was proposed in the article. A later publication by the same group [Boot *et al., J. Biol. Chem., 270*(44):26252-26256 (November 1995)] described the cloning of a human macrophage cDNA encoding a product that exhibited chitinase activity. See also International Patent Publication No. WO 96/40940, which reported two distinct human chitotriosidase cDNAs encoding a 50 kD and a 39kD product, both of which were fully enzymatically active. Renkema *et al., Eur. J. Biochem., 244*:279-285 (1997) reported that human chitinase is initially produced in macrophages as a 50 kD protein that is in part processed into a 39 kD form that accumulates in lysozymes, and also reported that alternative splicing generates a distinct human chitinase mRNA species encoding a 40 kD chitinase. Cloning of human chitinase cDNA was also reported in International Patent Publication No. WO 97/47752 (Gray).

Numerous immunoadhesins, which are chimeric molecules that combine the functional portion of a physiologically active protein with an immunoglobulin sequence, have been reported in the art, and are also known as "Ig-chimeras," "Ig-fusion proteins," "Fc-fusion proteins," and "receptor-globulins." See, *e.g.*, Harvill *et*

al., *Immunotechnology* 1:95-105 (1995) regarding IL-2 fusions and Zheng *et al.*, *J. Immunol*, 154:5590-5600 (1995) regarding IL-10 fusions; Fell, U.S. Patent No. 5,204,244. Although fusion of a biologically active molecule to an immunoglobulin sequence has been used to provide a longer half-life or to incorporate immunoglobulin functions such as Fc receptor binding, protein A binding, or complement fixation, such a fusion may be detrimental to the structure and biological activity of the functional molecule. The behavior of the ultimate fusion protein *in vivo* is thus not certain.

In view of the increasing incidence of life-threatening fungal infection in immunocompromised individuals, there exists a need in the art to identify new materials and methods useful for diagnosing and treating fungal infections.

SUMMARY OF THE INVENTION

The present invention relates to chitinase immunoglobulin fusion products comprising chitinase or fragments or variants thereof fused to at least a portion of an immunoglobulin chain. The invention provides novel purified and isolated polynucleotides (*i.e.*, DNA and RNA, both sense and antisense strands) encoding such fusion products; methods for the recombinant production of such fusion products; purified and isolated chitinase immunoglobulin fusion products; pharmaceutical and diagnostic compositions comprising such fusion products; and corresponding diagnostic or therapeutic uses, e.g., for detecting chitin, binding chitin, and treating fungal infections.

A presently preferred chitinase immunoglobulin fusion product comprises human chitinase fused at its C-terminus to the N-terminus of an IgG4 heavy chain constant region.

It is contemplated that the administration of a chitinase immunoglobulin fusion product for treatment of fungal infections may be accompanied by the concurrent administration of other non-chitinase antifungal therapeutic agents, including non-enzymatic antifungal agents.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows residual spleen fungal infection in mice challenged with sublethal doses of *C. albicans* and treated with varying amounts of rCH/Fc, amphotericin B or a combination of the two drugs.

Figure 2 shows residual kidney fungal infection in mice challenged with sublethal doses of *C. albicans* and treated with varying amounts of rCH/Fc, amphotericin B or a combination of the two drugs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to chitinase immunoglobulin fusion products comprising chitinase or fragments or variants thereof, preferably a human chitinase or fragments or variants thereof, fused to at least a portion of an immunoglobulin chain, preferably the constant region of a heavy chain. The invention is based upon the discovery that such fusion products retain chitinase biological activity, both *in vitro* and *in vivo*, have unexpectedly improved pharmacokinetics when administered *in vivo*, and have a decreased tendency to precipitate in buffer at protein concentrations of 1 mg/mL or more. As shown herein, fusion of human chitinase to an immunoglobulin constant region provides at least a 10-fold increase in the circulating serum half-life compared to the unfused human chitinase fragment alone. The fusion product retains not only the *in vitro* chitinase enzymatic and chitin-binding activities of unfused (or wild type) human chitinase, but also exhibits similar or improved *in vitro* synergy with other non-enzymatic antifungal agents in comparison to unfused human chitinase.

As used herein, "chitinase immunoglobulin fusion product" means a polypeptide comprising a chitinase product (*i.e.*, a chitinase or a fragment or variant thereof that retains chitinase activity), fused to at least a portion of an immunoglobulin (Ig) chain or a variant thereof. The Ig portion may be fused to either the N-terminus or C-terminus of the chitinase portion. Although human chitinase, fragments and variants thereof that retain chitinase and/or chitin-binding activity are most preferred, any chitinase may be used, including bacterial, insect and plant chitinases.

The cloning and expression of human chitinase cDNA and the

biological activities of recombinant human chitinase are described in detail in co-owned, co-pending U.S. Application Serial No. 08/877,599 filed June 16, 1997 and corresponding International Patent Publication No. WO 97/47752, which is a continuation-in-part of U.S. Application Serial No. 08/663,618 filed June 14, 1996, all of which are incorporated herein by reference in their entirety. The DNA sequence of the chitinase-coding insert of clone pMO-218 (deposited on June 7, 1996 under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, U.S.A., under Accession No. 98077) is set forth in SEQ ID NO: 1, and the encoded amino acid sequence is set forth in SEQ ID NO: 2. pMO-218 includes the entire coding region of the human chitinase cDNA (nucleotides 2 to 1399 of SEQ ID NO: 1), which comprises a twenty-one amino acid putative signal sequence followed by 445 encoded amino acids (residues 1 to 445 of SEQ ID NO: 2). The DNA sequence of the chitinase-coding insert of clone pMO-13B (deposited on June 7, 1996 under the terms of the Budapest Treaty with the ATCC under Accession No. 98078) is set forth in SEQ ID NO: 3 and the encoded amino acid sequence is set forth in SEQ ID NO: 4. This clone contains 25 additional nucleotides at the 5' end compared with MO-218; in addition, MO-13B (SEQ ID NO: 3) contains one different nucleotide at nucleotide position 330 (corresponding to nucleotide 305 of MO-218, SEQ ID NO: 1) which changes the encoded amino acid at position 80 of the mature protein from a glycine (in SEQ ID NO: 2) to a serine (in SEQ ID NO: 4).

The chitin-binding domain of human chitinase has been determined to reside in the C-terminal 49 amino acids of the protein, while triacetylchitotriose hydrolyzing activity has been observed to reside in the N-terminal portion (amino acids 1-373). See co-owned, co-pending U.S. Application Serial No. 09/267,574 filed March 12, 1999 and corresponding International Application No. PCT/US99/05343, which is a continuation-in-part of U.S. Application Serial No. 09/039,198 filed March 12, 1998, all of which are incorporated herein by reference.

The chitinase portion of the chitinase Ig fusion product may be a natural, recombinantly produced or wholly or partially synthetic chitinase or fragment or variant thereof that retains chitinase enzymatic and/or chitin-binding activity.

Variants may comprise chitinase analogs wherein one or more of the specified (i.e., naturally encoded) amino acids is deleted or replaced or wherein one or more nonspecified amino acids are added, without loss of the chitinase enzymatic and/or chitin-binding activity. It is contemplated that any conservative amino acid substitution known in the art may be made without affecting the biological activity of the variant. Fragments that retain chitin-binding activity are described in co-owned, co-pending U.S. application Serial No. 09/267,574 filed March 13, 1999, which is a continuation-in-part of U.S. application Serial No. 09/039,198 filed March 12, 1998, both of which are incorporated by reference herein.

In one embodiment, the human chitinase product component of the fusion protein comprises the mature human chitinase amino acid sequence set forth in SEQ ID NO: 2 or 4. In another embodiment, the human chitinase product component comprises a polypeptide encoded by: (a) a polynucleotide encoding the mature chitinase amino acid sequence set forth in SEQ ID NO: 2 or 4; or (b) a polynucleotide that hybridizes to the complement of the polynucleotide of claim (a) under stringent conditions. The human chitinase product may also comprise an amino acid sequence equivalent to (*e.g.*, at least about 99%, at least about 98%, at least about 95%, at least about 90%, at least about 85%, at least about 80% identical to) SEQ ID NO: 2 or 4 and include polypeptides with conservative substitutions to the amino acid sequence of SEQ ID NO: 2 or 4. "Conservative" substitutions of one amino acid for another are substitutions of amino acids having similar structural and/or chemical properties, and are generally based on similarities in polarity, charge, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues involved. Hydrophobic, polar neutral, polar basic and polar acidic amino acids may be grouped as described in Lehninger [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77], incorporated herein by reference. Hydrophobic amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (acidic) amino acids include aspartic acid and glutamic acid; basic amino acids include lysine, arginine and histidine.

The Ig portion of the chitinase Ig fusion product may be an entire Ig

chain, either heavy or light, but preferably is a fragment of the constant region of an Ig heavy chain that retains at least the CH2 and CH3 regions. The Ig chain is preferably from a human Ig and may be from any class of Ig, including IgG1, IgG2, IgG3, IgG4, IgM or IgA. However, an artificial consensus sequence rather than a natural Ig sequence may be used. In one embodiment, the chitinase is fused to a portion of human IgG4 that consists of two amino acids from the CH1 region and the hinge, CH2 and CH3 regions. Various deletions or substitutions of amino acids may be made within the Ig chain to produce variants that retain the improved half-life advantage of the Ig fusion product. For example, cysteine residues may be deleted or replaced with other amino acids, *e.g.* alanine or serine, to prevent disulfide crosslinking between Ig portions. The Fc receptor binding site and/or complement (C1q) binding site of the Ig constant region may also be modified by deletion or substitution of amino acid residues.

One aspect of the invention provides polynucleotides encoding such chitinase Ig fusion products (including natural, synthetic or partially synthetic DNA or RNA). DNA sequences which hybridize to the noncoding strand thereof under standard stringent conditions or which would hybridize but for the redundancy of the genetic code, and which encode polypeptides with chitinase and/or chitin-binding activity are also contemplated by the invention. Exemplary stringent hybridization conditions are as follows: hybridization at 42°C in 50% formamide and washing at 60°C in 0.1 x SSC, 0.1% SDS. It is understood by those of skill in the art that variation in these conditions occurs based on the length and GC nucleotide base content of the sequences to be hybridized. Formulas standard in the art are appropriate for determining exact hybridization conditions. See Sambrook *et al.*, 9.47-9.51 in *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

In another aspect, the invention includes biological replicas (i.e., copies of isolated DNA sequences made *in vivo* or *in vitro*) of DNA sequences of the invention. Autonomously replicating recombinant constructions such as plasmid and viral DNA vectors incorporating polynucleotides encoding chitinase Ig fusion products of the invention, including any of the DNAs described above, are provided. Preferred

vectors include expression vectors in which the incorporated fusion product-encoding cDNA is operatively linked to an endogenous or heterologous expression control sequence and a transcription terminator.

According to another aspect of the invention, procaryotic or eucaryotic host cells are stably transformed or transfected with polynucleotide sequences of the invention or otherwise genetically engineered (e.g., through homologous recombination) in a manner allowing the desired chitinase Ig fusion product to be expressed therein. Such host cells are particularly useful in methods for the large scale production of chitinase Ig fusion products wherein the cells are grown in a suitable culture medium permitting expression of the desired polypeptide and the desired polypeptide products are isolated, *e.g.*, by immunoaffinity or protein A purification from the cells or from the medium in which the cells are grown. Chitinase Ig fusion products may be partially or wholly chemically synthesized but are preferably produced by recombinant procedures involving host cells of the invention. The use of mammalian host cells may provide post-translational modifications (*e.g.*, myristolation, glycosylation, truncation, lipidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention.

Administration of chitinase Ig fusion products and therapeutic agents comprising such products to mammalian subjects, especially humans, for the purpose of ameliorating disease states caused by chitin-containing parasites such as fungi is contemplated by the invention. Fungal infections (mycoses) such as candidiasis, aspergillosis, coccidioidomycosis, blastomycosis, paracoccidioidomycosis, histoplasmosis, cryptococcosis, chromoblastomycosis, sporotrichosis, mucormycosis, and the dermatophytoses can manifest as acute or chronic disease and may be treated according to the invention. Treatment, either prophylactic or therapeutic, of fungal infection caused by a variety of fungal species is contemplated, including but not limited to the following: *Candida* species, including C. *tropicalis, C. parapsilosis, C. stellatoidea, C. krusei, C. parakrusei, C. lusitanae, C. pseudotropicalis, C. guilliermondi* and *C. glabrata*; *Aspergillus* species, including *A. fumigatus, A. flavus, A. niger, A. nidulans, A. terreus, A. sydowi, A. flavatus, A. glaucus, A. ustus,* and *A.*

versicolor; *Cryptococcus* species; *Histoplasma* species; *Blastomyces* species; *Coccidioides* species; *Paracoccidioides* species; *Fusarium* species; *Pneumocystis* species; *Entomophthorides* species; *Zygomycetes* and related species; *Basidiobolus; Conidiobolus; Rhizopus; Rhizomucor; Mucor; Absidia; Mortierella; Cunninghamella; Saksenaea; Pseudallescheria ,Sporotrichosis, Trichophyton, Trichosporon, Microsporum, Epidermophyton, Scytalidium, Malassezia, Actinomycetes, Penicillium, Saccharomyces; Sporothrix; Schenckii; and Chromomycosis* species, e.g. *Fonsecaea pedrosoi, Fonsecaea compacta.*

Pathogenic fungi cause serious, often fatal disease in immunocompromised subjects, and products of the invention are expected to be particularly helpful in treating infections in these subjects. Cancer patients undergoing chemotherapy, immunosuppressed individuals, and HIV-infected individuals are susceptible to mycoses caused by *Candida*, *Aspergillus*, *Pneumocystis carinii*, and other fungi. Amphotericin B and fluconazole are useful therapeutics for fungal infections, but toxicity associated with these drugs causes serious adverse side effects that limit their usefulness. The mortality of systemic candidiasis is greater than 50% despite amphotericin B treatment.

Thus, it is contemplated that the chitinase Ig fusion products may be concurrently administered with or even conjugated to other conventional (preferably non-enzymatic) antifungal agents, including amphotericin B and the structurally related compounds nystatin and pimaricin; 5-fluorocytosine; azole derivatives such as fluconazole, ketoconazole, clotrimazole, miconazole, econazole, butoconazole, oxiconazole, sulconazole, terconazole, itraconazole and tioconazole; allylamines-thiocarbamates, such as tolnaftate, naftifine and terbinafine; griseofulvin; ciclopirox olamine; haloprogin; undecylenic acid; and benzoic acid. [See, *e.g.*, Goodman & Gilman, The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, NY (1996).] According to this aspect of the invention, the chitinase Ig fusion products are expected to improve the effectiveness of these conventional antifungal agents, perhaps by rendering the fungi more susceptible to their action. A reduction in the amount of conventional antifungal agent needed to exert the desired antifungal effect may allow the drugs to be used at less toxic levels. This effect of the chitinase Ig fusion product

on the activity of conventional antifungal agents can manifest as classic checkerboard synergy in *in vitro* tests as indicated, *e.g.* by the fractional inhibitory concentration (FIC) index or the fractional fungicidal concentration (FFC) index, or may manifest as conversion of a fungistatic agent into a fungicidal agent or as a reduction in the resistance of fungi to conventional antifungal agents.

Conjugation to non-selective cytotoxic agents may allow selective targeting of these agents through the chitin-binding function of the chitinase Ig fusion products. Alternatively, chitinase Ig fusion products may be administered alone since the products themselves may have antifungal effects. Multimeric chitinase Ig fusion products may provide enhanced antifungal effects and include multimeric products that have been covalently cross-linked by chemical means; recombinantly produced polypeptides comprising multiple chitinase portions linked in tandem with the Ig portion; and dimeric or multimeric chitinase Ig fusion products linked through disulfide bonding of the Ig portions. The administration of multimeric products may also reduce the amount of concurrently administered conventional antifungal agent necessary to exert a desired antifungal effect.

Thus, the invention contemplates the use of chitinase Ig fusion products in the preparation of a medicament for the prophylactic or therapeutic treatment of fungal infections.

Specifically contemplated by the invention are therapeutic/pharmaceutical compositions comprising chitinase Ig fusion products for use in methods for treating a mammal, preferably a human, susceptible to or suffering from fungal infections. Such compositions may include a physiologically acceptable diluent or carrier, and may also include other conventional antifungal agents. Dosage amounts indicated would be sufficient to supplement endogenous chitinase activity. For general dosage considerations see *Remington: The Science and Practice of Pharmacy*, 19th ed., Mack Publishing Co., Easton, PA (1995). Dosages will vary between about 1 μg/kg to 100 mg/kg body weight, and preferably between about 0.1 to about 20 mg chitinase Ig fusion product/kg body weight. Therapeutic compositions of the invention may be administered by various routes depending on the infection to be treated, including via subcutaneous, intramuscular, intravenous, intrapulmonary,

transdermal, intrathecal, topical, oral, or suppository administration.

"Concurrent administration" as used herein includes administration of the different agents together, or before or after each other. The different agents may be administered by the same or different routes. For example, one agent may be administered intravenously while the second agent is administered intramuscularly, intravenously, subcutaneously, orally, topically or intraperitoneally. The different agents may be administered simultaneously or sequentially, as long as they are given in a manner sufficient to allow all agents to achieve effective concentrations at the site of infection.

Example 1 addresses construction of a vector encoding a human chitinase immunoglobulin (Ig) fusion product. Example 2 addresses expression of this vector in COS cells. Example 3 addresses purification and formulation of a human chitinase Ig fusion product. Examples 4, 5 and 6, respectively, demonstrate chitinase enzymatic activity, chitin-binding activity and pharmacokinetics of a human chitinase Ig fusion product. Examples 7 and 8, respectively, demonstrate *in vitro* and *in vivo* antifungal activity of a human chitinase Ig fusion product when administered concurrently with other antifungal agents. Example 9 addresses construction of a vector encoding a human chitinase Ig fusion product for expression in CHO cells.

EXAMPLE 1

Construction of vector encoding human chitinase Ig fusion product

Plasmids pMO218 (ATCC Accession No. 98077) and pMO13B (ATCC Accession No. 98078) each contain complete human chitinase cDNA and are described in co-owned, co-pending U.S. Application Serial No. U.S. Application Serial No. 08/877,599 filed June 16, 1997 and corresponding International Patent Publication No. WO 97/47752, both of which are incorporated herein by reference in their entirety. Vectors encoding a human chitinase immunoglobulin fusion product (designated rCH/Fc and consisting of essentially full length human chitinase fused at its C-terminus to the N-terminus of the constant region of human IgG4) were constructed from plasmids pMO218 and pMO13B as follows.

The C-terminus of the human chitinase cDNA was modified by PCR to delete the termination codon and replace it with a *XhoI* restriction site. Primers 218-4 (5' TTCAACAGTGGCTGCAGA 3', SEQ ID NO: 5), which binds upstream of a unique SphI site at about nucleotide 741 of human chitinase, and 713XhoI (5' TACACTCGAGATTCCAGGTGCATTTGC 3', SEQ ID NO: 6), which deletes the putative termination codon of CH and adds the XhoI restriction site, were used to amplify the C-terminal portion of human chitinase. A 50 μL PCR reaction was carried out with the following reagents: 5 μL of 10X PCR salt (0.5 M KCL, 100 mM Tris, pH 8.3, 15 mM $MgCl_2$); 5 μL of 5 mM dNTP's; 5 μL of primer 218-4 at 100 ng/mL; 5 μL of primer 713XhoI at 100 ng/mL, 1 μL of DNA template (plasmid CH218, also referred to as MO218) diluted 1:50 in TE, pH 8.0); 0.5 μL of TAQ1 polymerase and 30 μL of H_20. The reagents were mixed together, overlaid with 100µL of mineral oil and amplified at 94 °C for 4 minutes, followed by one minute at 94 °C, 1 minute at 60 °C, and 1 minute at 72 °C for 31 reaction cycles on a Perkin Elmer Cetus DNA Thermal Cycle machine. The resulting PCR product was purified over a CL6B resin (Pharmacia) to remove excess primer and any primer dimers.

A 607bp *SphI/XhoI* fragment of this PCR product encoding the modified C-terminal portion of human chitinase was then joined in a three-way ligation, described below, with an 872bp *HindIII/SphI* DNA fragment encoding the N-terminal portion of human chitinase (including the leader sequence) and the *HindIII/XhoI*-digested vector pDEF2S/Ig4 which encodes the immunoglobulin constant region of human IgG4.

Ten μL of the purified PCR product encoding the modified C-terminal portion of human chitinase was digested in a 20 μL reaction with 2 μL of 10X salt and 1 μL each of restriction enzymes *SphI* and *XhoI*. After incubation for one hour at 37°C, a 607-bp fragment was isolated.

The 872bp *HindIII/SphI* DNA fragment was obtained as follows. Approximately 2 μg of each of pMO218 and pMO13B was digested with *HindIII* and *SphI* in a 20 μL reaction that contained 2 μL of 10X high salt buffer (Boehringer Mannheim (BMB)), 1 μL each of restriction endonucleases *HindIII* (BMB 10U/μl) and *SphI* (BMB 10 U/μL) and 13 μL of H_2O. After incubating 1 hour at 37 °C, an

872-bp fragment encoding the N-terminal portion of human chitinase was isolated for each plasmid.

pDEF2S/Ig4 was produced as follows. The plasmid pDEF2S was first constructed from pDEF2 (described in US Pat. No. 5,888,809) by digesting pDEF2 with *SalI*, followed by blunting with Klenow polymerase and reclosure of the plasmid with T4 DNA ligase, thereby destroying the *SalI* site. The plasmid pDEF2S/Ig4 was constructed from pDEF2S by cloning into the *HindIII-XbaI* site a 1217 bp *HindIII - XbaI* fragment with the sequence in SEQ ID NO: 7. This fragment contains: a *XhoI* restriction site, immediately followed by codons corresponding to the final two amino acids of the human immunoglobulin g4 heavy chain CH1 domain, immediately followed by cDNA sequence encoding the hinge, CH2, and CH3 regions of the human g4 protein, immediately followed by 330 bp of sequence that is naturally present in the 3' flanking region of the human g4 gene and which contains a polyadenylation signal [Ellison et al., DNA, 1:11-18 (1981)], followed, 25 bp downstream, by a *XbaI* restriction site.

Approximately 2 μg of the resulting plasmid pDEF2S/Ig4 was digested in a 20 μL reaction with 2 μL 10X salt and 1 μL each of restriction enzymes HindIII and XhoI. After incubation for one hour at 37°C, 2.2 μL of calf intestinal phosphatase buffer [Sambrook *et al.*, *Molecular Cloning*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)] was added to the reaction mixture along with 0.5 μL of alkaline phosphatase (BMB) and the mixture was further incubated at room temperature for 15 minutes.

Fragments were isolated after each of the three digestion reactions by adding 4 μL of 5X DNA loading dye to each sample, loading 15 μL onto a 0.8% low melting agarose [LMP agarose, BRL] 1X TAE agarose gel, electrophoresing at 90m Amps for about 30 minutes or until the dye front had migrated over greater than half of the gel, and removing each band with a razor blade.

The three fragments were briefly heated to 65°C (for 5 minutes) before being ligated at room temperature (~22°C) for one hour in a reaction mixture containing: 4 μL of the *HindIII/SphI* (N-terminal) fragment from either the MO218 or MO13B plasmid, 4 μL of the PCR amplified *SphI/XhoI* (C-terminal) fragment, 2 μL of

CIP-treated pDEF2S/Ig4 *HindIII/XhoI* fragment, 2 μL of 10X ligase salt (BMB) and 1 μL of T4 DNA ligase (BMB). After the ligation reaction was complete each reaction was briefly heated to 65μC and 15 μL of the ligation mixture was added to 100 μL of $CaCl_2$ competent *E. coli* cells. Cells were incubated on ice for 30 minutes, heat shocked for 2 minutes at 42°C and plated onto Luria Broth with Mg^{2+}/agarose plates containing 100 μg/mL of carbenicillin. Plates were incubated overnight at 37°C. Single colonies were transferred to 3.0 mLs LBM containing 100 μg/mL carbenicillin and cultured overnight at 37°C. The plasmid DNA was isolated from the cells using the Wizard™ miniprep kit. (Promega, Madison, WI) and was tested for the presence of an insert by restriction digestion. Five μL of plasmid DNA was digested in a 20 μL reaction containing 2 μL of 10X salt, 12 μL H_2O and 1μL each of restriction endonucleases *HindIII* and *XhoI*, incubated at 37°C for about one hour, then loaded on a 0.8% agarose 1X TAE gel. Digestion reactions that yield a band of the proper size, approximately 2.5 kb, were assumed to contain the proper insert. Miniprep DNA preparations designated nos. pDEF2S/CH13B/Ig4-1 through -10 (or 13B-1 through 13B-10) represented 13B/Ig4 isolates and miniprep DNA preparations designated nos. pDEF2S/CH218/Ig4-11 through -20 (or 218-11 through 218-20) represented 218/Ig4 isolates. Plasmids 13B-1, 13B-10, 218-13 and 218-17 were sequenced to confirm that the plasmids were free of PCR errors that would change the amino acid sequence of the protein.

EXAMPLE 2

Production of human chitinase Ig fusion product in COS cells

Plasmids pDEF2S/CH218/Ig4-13 (or pCH218/Ig4) and pDEF2S/CH13B/Ig4-1 (or pCH13B/Ig4) were transiently transfected into COS cells as follows using DEA dextran chloroquine method, and supernatant containing the recombinant protein was collected and assayed for chitinase enzymatic activity and for protein concentration as determined by ELISA.

COS cells were split 1:5 prior to transfection such that they would be between 40-50% confluent on the day of transfection. On the day of tranfection each plate was washed with 10 mLs of PBS. A master mix containing 20 μg of plasmid

pCH/Ig4 DNA, 62.5 μL of DEA- Dextran (50 mg/mL, Sigma), 2.5 μL of chloroquine (0.25M, Sigma) and 6.25 mLs of serum free DMEM was added to a 15.0 mL conical tube. The master mix was then added to the cells and incubated for 1.5 hours at 37°C. After 1.5 hours, tranfection media was aspirated and replaced by 6 mLs of 10% DMSO in PBS for about one minute. The cells were then washed in 10 mLs of phosphate buffered saline (PBS) and were incubated overnight in DMEM plus 10% FBS. The following day the cells were washed twice with 10 mLs PBS, and 10 mLs of serum free media was added to the plate. Cells were further incubated for three days at 37°C. Media was collected, purified as described in Example 3 below, and chitinase enzymatic activity, chitin-binding activity, pharmacokinetics and *in vitro* and *in vivo* activity of the purified product, called rCH/Fc, was determined as described in Example 4 to 8 below.

EXAMPLE 3

Purification and formulation of human chitinase Ig fusion product

The COS cell supernatant containing the recombinant fusion product was purified as follows over a protein A column in a single step purification procedure that yielded > 90% pure protein. Briefly, the supernatant (about 9 mLs) was concentrated over a 30K centrifugal filter column (UFV2BTK40 Millipore Corp., Bedford, MA) to one mL. The concentrated media was then loaded at 0.5-1.0 cm/min onto a Protein A Sepharose FF column (Pharmacia) that had been equilibrated with 1X CMF-PBS (2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, 8.1 mM Na_2HPO_4). The column was washed with equilibration buffer, CMF-PBS, until the A_{280} profile returned to baseline. Then the column was washed with 20 mM phosphate, pH 7.2. This low ionic strength wash was necessary prior to elution at a low pH (3.5) because concentrated human chitinase precipitates at low pH (<4.5) in the presence of salt. The human chitinase Ig fusion product was eluted with 40 mM citrate, pH 3.5, into fraction tubes containing 1/10th volume of 1 M Na phosphate, pH 8.0, as a neutralizing buffer, and the peak eluate was pooled and dialysed in CMF-PBS. The column may then be stripped with 100 mM citrate, pH 3.0, to remove any remaining protein (which is not used). The resulting purified chitinase immunoglobulin fusion product was

designated rCH/Fc.

The concentration of fusion product present in the rCH/Fc samples can be determined by quantifying the human IgG portion of the fusion using an ELISA. Microtiter plates (Immulon 4, Dynatech) were coated overnight at 2-8°C with 50 μL/well of 1:1000 goat anti-human IgG (Jackson ImmunoResearch Cat. No. 109-005-003) in 50 mM $NaHCO_3$, 0.01% NaN_3, pH 9.6. Coating solution was aspirated and plates were washed three times with 350 μL of wash buffer (CMF-PBS, 0.05% Tween 20). Assay standards (5-100 ng/mL) were prepared from a stock solution of a control humanized monoclonal IgG4 diluted in Dulbecco's Modified Eagle's Medium (DMEM)/F12 containing 10% dialyzed fetal bovine serum, 2 mM L-glutamine, 100 μ/mL penicillin, and 0.1 mg/mL streptomycin. Samples were tested at four dilutions in media: an initial dilution of 1:20 or 1:40, with three subsequent serial 5-fold dilutions. Standards and samples (50 μL/well) were incubated in anti-human IgG-coated wells for 90 minutes at room temperature or for 30 minutes at 37°C. After aspirating samples, plates were washed three times with wash buffer. Goat antihuman Fc fragment specific-HRP (Jackson ImmunoResearch Cat. No. 109-035-098), 50 μL of a 1:10,000 dilution in wash buffer, was added per well and incubated for 60 minutes at room temperature or for 30 minutes at 37°C. Plates were washed again and 100 μL/well of 1:100 tetramethylbenzidine (TMB, 10 mg/mL in 100% DMSO, Sigma T 2885) in buffered substrate (0.1 M sodium acetate, 0.015% H_2O_2 pH 5.5) was added and incubated for 30 minutes at room temperature in the dark. The reaction was stopped by the addition of 50 μL of 15% H_2SO_4 and the absorbance at 450 nm (A_{450}) was determined.

The data for standards were fitted to a polynomial equation. The concentration of IgG in the test samples was determined using the curve-fit equation, the A_{450}, and the sample dilution.

The rCH/Fc product could be readily formulated in CMF-PBS (2.7 mM KCl, 1.5 mM KH_2PO_4, 137 mM NaCl, 8.1 mM Na_2HPO_4, pH 7.4) at concentrations as high as 10 mg/mL. In contrast, unfused (wild type) human chitinase, which was formulated in 20 mM Tris, pH 7.5, 150 mM NaCl, 5% glycerol, was difficult to formulate because even 1 mg/mL concentrations precipitated out of solution.

EXAMPLE 4

Chitinase enzymatic activity assay

To evaluate chitinase enzymatic activity in a 96-well microtiter plate format on a Beckman BioMek 1000 robotic station. Serum samples to be tested were initially diluted 1:1000 in dilution buffer (0.2 M sodium acetate, 0.1% BSA, 0.05% ProClin 300, pH 5.2) followed by four serial 4-fold dilutions. 70 mL aliquots of each dilution were transferred to the wells of a Wallac 96-well sample plate and 70 mL of fluorogenic substrate, 100 mM 4-methylumbelliferyl b-D-N, N', N"-triacetylchitotrioside (Sigma M 5639) in dilution buffer, was added to each well. The plate was incubated at 50°C for one hour. The reaction was stopped with 70 mL/well of stop buffer (0.6 M sodium carbonate). The plates were read in a Wallac Victor plate reader at an excitation wavelength of 355 nm and emission wavelength of 460 nm. Assay standards comprised 70 mL aliquots of the fluorescent reaction product 4-methylumbelliferone (Signma M 1381), over the concentration range of 0.20-50 nM in 4-fold increments. Standards were tested in duplicate. A standard curve was constructed by fitting the fluorescence units versus standard concentration data to a straight line. The chitinase activity in the sample was determined in the linear response region for each sample and expressed as nmol/min/mL.

In this assay, the rCH/Fc product obtained as described in Example 3 above had a specific activity of approximately 25-32 μg/min/mg.

EXAMPLE 5

Chitin-binding activity assay

The chitin binding activity of rCH/Fc was evaluated as follows. 200 μg of rCH13B/Fc or rCH218/Fc were diluted to 500 mL with equilibration buffer (20 mM Tris pH 8.0, 500 mM NaCl). A chitin column was prepared with 500 mL of chitin beads (New England Biolabs) in a minicolumn. After the beads settled, the column was washed with 5 mL of 1% SDS followed by 5 mL of equilibration buffer. The test sample was added to the column and the flow through fraction was collected. The column was then washed with 5 mL of equilibration buffer and two fractions (the first 1 mL and the remaining 4 mL) were collected. The chitin-bound molecules were

eluted from the column with 2 mL of 1% SDS in two fractions (the first 1 mL and the remaining 1 mL). All of the fractions were run on a 12% polyacrylamide gel, and results showed that a band of the molecular weight expected for rCH/Fc was observed only in the eluted fractions and not in the wash fractions, indicating that the rCH/Fc bound to the chitin beads in the column.

EXAMPLE 6

Pharmacokinetics of human chitinase Ig fusion product

The pharmacokinetics of the human chitinase Ig fusion product produced as described in Examples 1-3 above was determined as follows. Balb/C mice received a single intravenous injection into the tail vein of 5.0 mg/kg of this fusion product. At 0, 0.5, 1, 2, 4, 6, 8, 16 and 24 hours after injection, whole blood was collected by cardiac puncture and placed in Microtainer® (Beckton-Dickinson) red cap tubes for serum collection by centrifugation at 6000 rpm for 2 or 3 minutes. Urine was also collected at the same time points. Samples were placed in Eppendorf ® tubes and refrigerated at 4-6°C until all time points were collected. Enzymatic activity was determined for each sample as described above in Example 4. Protein concentration was also determined for each sample as derived from the A_{280} values and extinction coefficient. The serum half-life of the CH/Ig4 fusion was determined to be about 29.9 hours using the Winonlin model #1.

The experiment was repeated with male 6-8 week old CD-1 mice (5 per time point) administered a dose of 77 mg/kg rCH/Fc (using a solution of 11.24 mg/mL rCH/Fc) by either an intravenous or an intraperitoneal route. Chitinase activity and protein concentration were assayed in serum samples collected via cardiac puncture at 0, 1, 2, 4, 6, 8, 16, 24, 28, 32 and 48 hours after injection. The resulting pharmacokinetic data are set forth below in Table 1.

Table 1

Dose (mg/kg)	AUC (μg/mL/h)	cL (mL/h/kg)	MRT (h)	half-life (h)	Cmax (μg/mL)
77 (IV)	22800	3.4	42	28	839
77 (IP)	17500	4.4	32	19	491

AUC: area under serum-concentration-time curve extrapolated to infinity
cL: total body clearance
MRT: mean residence time
half-life: serum half-life for terminal elimination phase
Cmax: maximum observed serum concentration

Pharmacokinetic data for wild type human chitinase, determined in a previous study, are set forth below in Table 2. Female Balb/c mice, 6-8 weeks old, were injected intravenously in the tail vein with 0.5 mg/kg, 5.0 mg/kg and 50 mg/kg recombinant human chitinase. For each dose group, mice were terminally bled at 0.01, 0.25, 1, 8 and 24 hours after injection (2 animals were used per time point per dosage) and serum samples were assayed for chitinase activity and concentration.

Table 2

Dose (mg/kg)	AUC (μg/mL/h)	Vss (mL/kg)	cL (mL/h/kg)	MRT (h)	half-life (h)	Cmax (μg)
0.5	31.24	12.03	16.01	0.75	0.74	22.30
5.0	278.50	13.61	17.95	0.76	1.38	162.84
50.0	2505.83	52.92	19.95	2.65	2.33	1179.19

AUC: area under curve to time infinity
Vss: steady state volume of distribution
cL: clearance
MRT: total body mean residence time
Cmax: peak serum concentration

The rCH/Fc product thus was observed to have an at least 10-fold increase in the serum half-life in comparison to the half-life previously determined for wild type human chitinase.

EXAMPLE 7

In vitro antifungal activity of human chitinase Ig fusion product

In vitro antifungal susceptibility testing of various fungi was carried out to determine the effect of rCH/Fc alone and in combination with other conventional antifungal agents, such as amphotericin B. Classic "checkerboard" assays were performed by (1) inoculating a checkerboard of tubes with a standardized broth suspension of fungal cells, (2) adding serial dilutions of the first test compound (*e.g.*, human chitinase) to all rows of the checkerboard and (3) adding serial dilutions of the second test compound (*e.g.*, amphotericin B) to all columns of the checkerboard. Minimum inhibitory concentrations (MICs), *i.e.*, the lowest concentration of test compound which produces no visible growth (a clear tube), and minimum fungicidal concentrations (MFCs), *i.e.*, the lowest concentration of test compound which gives greater than 96% killing upon subculture, were determined for each test compound, alone and in combination. These values were then used to calculate the fractional inhibitory concentration (FIC) index and the fractional fungicidal concentration (FFC) index, which are values that indicate the extent to which the first test compound reduces the amount of the second test compound needed to exert the desired inhibitory or fungicidal effect. An FIC or FFC index of less than 1 is considered to show synergy, while an FIC or FFC index of 0.5 or less is considered to show potent synergy. "Indifference" means that the first test compound does not affect the activity of the second test compound at all (*i.e.*, the full MIC or MFC is required).

Results of testing the combinations of rCH/Fc with amphotericin B (AMB) on various clinical isolates of *Cryptococcus neoformans* are displayed in Table 3 below.

Table 3

Organism and Strain	Drug No. 2	Human chitinase		Drug No. 2		FIC index	FFC index
		MIC (μg/ml)	MFC (μg/ml)	MIC (μg/ml)	MFC (μg/ml)		
C. neoformans 97-59	AMB	> 64	> 64			≤ 0.27	≤ 0.28
C. neoformans 98-19	AMB	> 64	> 64			≤ 0.16	≤ 0.25
C. neoformans 97-21	AMB	16	> 64	1.0	4.0	0.5	≤ 0.25

The rCH/Fc product alone showed activity against the *C. neoformans* 97-21 isolate, with an MIC of 16 μg/mL that was a slight improvement over the MIC of 32 μg/mL for wild type human chitinase (determined in previous studies using the 97-21 isolate).

When tested in combination with amphotericin B, rCH/Fc showed quite potent synergy with amphotericin B on all of the *C. neoformans* strains, for both growth inhibition and for killing.

EXAMPLE 8

In vivo antifungal activity of human chitinase Ig fusion product

The antifungal activity of rCH/Fc, both alone and in combination with amphotericin B, was tested in a murine model of systemic candidosis in which the mice were challenged with sublethal doses of fungi. The 6 week old male CD-1 mice (average weight 29.6 g) were infected by intravenous inoculation with 9×10^4 CFU of a clinical isolate of *Candida albicans*. Treatment began four days after infection. Groups of 10 mice received either phosphate buffered saline (diluent controls) administered intraperitoneally; rCH/Fc (in PBS in a volume of 0.53 ml) administered intraperitoneally at a dose of 200, 80 or 20 mg/kg once daily for 12 days; amphotericin B (AmB, PharmaTek, in sterile 5% dextrose in a 0.2 mL volume) administered intraperitoneally at a dose of 3 mg/kg three times weekly for 2 weeks at days 4, 6, 8,

11 13 and 15 post infection (6 doses total); or both AmB and rCH/Fc. Another control group of 10 mice received no fungal challenge and the 200 mg/kg rCH/Fc treatment regimen.

Deaths were tallied through 18 days of infection, 3 days after cessation of therapy. On day 18 all surviving infected mice were euthanized with carbon dioxide gas and the number of CFU of *C. albicans* remaining in the spleen and kidneys was determined by culturing 10-fold dilutions of homogenized organ tissue.

The rCH/Fc-treated groups showed no significant difference in mortality or residual spleen or kidney fungal load compared to untreated controls. The AmB and rCH/Fc combination-treated groups showed no significant difference in mortality but showed a dramatic reduction in residual spleen fungal infection. All survivors of the group given AmB and 200 mg/kg rCH/Fc were free of residual infection in the spleen, and survivors of the group given AmB and either 80 or 20 mg/kg rCH/Fc were combinations were 90% free of residual spleen infection. In comparison, all survivors of the groups treated with AmB alone showed some residual spleen infection. When the combination treatments were compared to AmB alone, a statistically significant reduction in spleen fungal load was observed for all dosages of rCH/Fc ($p=0.01$ for 200 mg/kg rCH/Fc+AmB vs. AmB alone; $p=0.001$ for 80 mg/kg rCH/Fc+AmB vs. AmB alone; $p=0.001$ for 20 mg/kg rCH/Fc+AmB vs. AmB alone). The combination treatment with AmB and rCH/Fc at these doses resulted in an apparent increased residual kidney fungal load compared to the group treated with AmB alone, suggestive of potential antagonism in the kidney.

When the experiments were repeated with lower doses of rCH/Fc (0.2, 2 and 20 mg/kg) the results confirmed that the AmB and rCH/Fc combination treatment dramatically reduced residual spleen fungal infection, even at the lowest (0.2 mg/kg) dose of rCH/Fc tested. When the combination treatments were compared to AmB treatment alone, a reduction in spleen fungal load was observed for all dosages of rCH/Fc; the reduction reached statistical significance for dosages of 2 mg/kg rCH/Fc ($p=0.0013$ vs. AmB alone) and 0.2 mg/kg rCH/Fc ($p=0.0022$ vs. AmB alone). The results are graphically displayed in Figure 1. The previously observed increase in residual kidney fungal load for the combination treatment (compared to AmB alone)

was not observed at these doses of rCH/Fc. See Figure 2.

EXAMPLE 9

Construction of a vector for CHO cell expression of Ig fusion product

Plasmids for expression of human chitinase Ig fusion product in CHO cells, pDEF24/CH13B/Ig4 or pDEF24/CH218/Ig4, were constructed as follows. Plasmids pDEF2S/CH13B/Ig4-1 and pDEF2S/CH218/Ig4-13 were digested in a 20 μL reaction containing approximately 2 μg DNA, 2 μL of 10X salt, 1 μL each of the restriction endonucleases *NotI* and *XbaI* and 12 μL of H_2O. Each reaction digest was incubated at 37°C for one hour and DNA fragments were isolated on a 0.8% LMA 1X TAE gel. The *NotI/XbaI* fragment containing the chitinase gene was ligated with a *NotI/XbaI* fragment from a pDEF24-derived vector (pDEF30) treated with alkaline phosphatase (CIP) as described above in Example 1, and the ligation products were used to transform $CaCl_2$ competent cells as described above in Example 1. The pDEF30 vector is the same as pDEF24 except that it includes a 321bp fragment from pDC1 (described in U.S. Patent No. 5,888,809, incorporated herein by reference) containing a BGH polyA region. In turn, pDEF24 was derived from pDEF14 (also described in U.S. Patent No. 5,888,809) by digesting pDEF14 with *AscI* and *BsiWI*, removing the 2324 bp fragment containing 5' sequence flanking the SHEF1 promoter, blunting with Klenow, and religating. DNA from transformants was isolated using the Wizard™ miniprep kit (Promega) and digested with *NotI* and *XbaI*. Plasmids that contained an insert of the appropriate size, approximately 3.3 kb, were assumed to be correct. Plasmids pDEF24/13B/Ig4-1 through -6 and pDEF24/218/Ig4-7 through 12 were transfected into CHO cells; of those, pDEF24/218/Ig4-11 was selected for CHO cell expression of the fusion protein.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the present invention are those which appear in the appended claims.

WHAT IS CLAIMED IS:

1. A chitinase immunoglobulin fusion product comprising a human chitinase product having the mature human chitinase amino acid sequence set forth in SEQ ID NO: 2 or 4 fused to at least a portion of an immunoglobulin chain.

2. A chitinase immunoglobulin fusion product comprising a human chitinase product fused to at least a portion of an immunoglobulin chain, said human chitinase product comprising a polypeptide encoded by:

(a) a polynucleotide encoding the mature chitinase amino acid sequence set forth in SEQ ID NO: 2 or 4; or

(b) a polynucleotide that hybridizes to the complement of the polynucleotide of claim (a) under stringent conditions.

3. The chitinase immunoglobulin fusion product of claim 2 wherein said human chitinase product is fused to a constant region of a human IgG heavy chain or portion thereof.

4. A polynucleotide encoding the chitinase immunoglobulin product of claim 1 or claim 2.

5. The polynucleotide of claim 4 which is DNA.

6. A vector comprising the DNA of claim 5.

7. A host cell transformed with the DNA of claim 5.

8. A method of producing a chitinase immunoglobulin fusion product comprising culturing the host cell of claim 7 in growth medium and isolating from said host cell or its growth medium said chitinase immunoglobulin fusion product.

9. A pharmaceutical composition comprising the chitinase immunoglobulin

fusion product of claim 1 or claim 2.

10. A method of treating a fungal infection comprising the step of administering to a subject an antifungal amount of a chitinase immunoglobulin fusion product.

11. The method of claim 10 further comprising the step of administering an antifungal amount of a non-chitinase antifungal agent.

12. A method of reducing the amount of a non-chitinase antifungal agent needed to exert an antifungal activity in a subject, comprising administering to said subject an amount of a chitinase immunoglobulin fusion product effective to improve the antifungal activity of said antifungal agent.